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SHORT PAPER

A simple technique for the identification of chain termination suppressor mutants in species of *Salmonella*

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SUMMARY

Chain-termination suppressors which are almost certainly amber suppressors have been isolated in *Salmonella anatum* by a technique involving the use of amber mutants of bacteriophage 01. The same technique could be used in any species of *Salmonella* (and many strains of *Arizona*) and analogous techniques are suggested for use in other genera of bacteria and in higher plants.

Bacteriophage ϵ^{15} is capable of both generalized (low-frequency) transduction (Iseki & Sakai, 1954) and specific (high-frequency) transduction of a variety of genetic markers, both plasmid borne (Kameda *et al.* 1965) and chromosomal (Hedges, 1971). In order to study the organization of the transducing elements of this phage it was essential to accumulate a set of conditional lethal mutations. Temperature-sensitive mutants have disadvantages for this type of study, notably their tendency to exhibit intragenic complementation (Bernstein, Edgar & Denhardt, 1965; Edgar & Lielausis, 1964), whereas chain-termination mutants normally do not permit this (Edgar, Denhardt & Epstein, 1964). Three triplets have been identified as translation terminators in bacteria and suppressors of all of these have been reported in strains of *Escherichia coli* (Brenner, Stretton & Kaplan, 1965; Brenner *et al.* 1967) and in *Salmonella typhimurium* LT 2 (Berkowitz *et al.* 1968). The best studied class of chain-terminating mutants consists of amber (UAG) mutants. Amber-suppressor mutants permit efficient chain extension beyond the amber mutation site, whilst ochre suppressors are much less efficient (Stretton, Kaplan & Brenner, 1967). For this reason, amber mutants are very convenient for many studies.

Phage ϵ^{15} grows on Salmonellae of subgenus E 1 (Uetake, Nakagawa & Akiba, 1955) the preferred host being *S. anatum* strain A (Uetake, Luria & Burrous, 1958). No strains of a suitable host bacterium capable of suppressing chain-terminating mutations were available, so it was necessary to devise a technique for the isolation and identification of such a strain. The chosen technique is described in this paper since it may prove to be of general utility.

Phage 01 (Felix & Callow, 1943) is remarkable for being able to grow on almost any strain of *Salmonella* (and on many strains of *Arizona*) (Cherry, Davis, Edwards & Hogan, 1954). A stock of phage 01 was prepared on a strain of *S. typhimurium* LT 2. It gave equal plaque counts on *S. typhimurium* LT 2 and on *S. anatum* A 1, which indicates that phage 01 must be non-susceptible to the restriction system of *S. anatum* A 1 (Uetake, Toyama & Hagiwara, 1964). A number of coliphages are known to be unaffected by particular restriction systems (Eskridge, Weinfeld & Paigen, 1967).

Some strains of *S. typhimurium* LT 2 carry amber-suppressor mutations. Two such strains, isogenic except that one carried the amber suppressor (sup 711) whilst the other lacked

suppressor activity, were used in these studies. These strains, supplied by Drs Atkins & Ryce of Trinity College, Dublin, Eire, carry the amber histidine mutant *his* D873. Four independently arising lines of phage 01 capable of forming plaques on the suppressor-carrying strain but not on the strain lacking suppressor activity were isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG). These must be amber mutants since the suppressor is specific for amber mutants (J. F. Atkins, personal communication).

Of these four mutants, two were rather unstable (reversion rates $> 10^{-5}$), whilst the other two had reversion rates around 10^{-6}. Only the latter two strains, *am 1* and *am 4*, were used in these studies. They could be used to identify suppressor-carrying strains of any species of *Salmonella*. These mutant phages, capable of absorbing to many strains of *Salmonella* but to form plaques only upon those strains containing amber-suppressor mutants (and perhaps strains carrying ochre-suppressor mutants) (Stretton *et al.* 1967) were used to identify suppressor-carrying mutants of *S. anatum* A1. Since the mutant phages cannot reliably be used to distinguish between bacterial strains carrying amber suppressors and those carrying ochre suppressors, it was desirable to use a technique that selected amber rather than ochre suppressor strains.

Auxotrophic mutants were selected by screening after mutagenesis with NG. It is known that a high proportion of the nonsense mutants induced by this mutagen are amber mutants and that very few are ochre mutants (Zipser, 1967).

Whitfield, Martin & Ames (1966) have reported that, among the *hisC* mutants of *S. typhimurium*, amber have an average reversion frequency of about 5×10^{-7} (excluding a single very unstable mutant) whereas ochre mutants have a reversion rate of about 2×10^{-8}. In order to increase the proportion of amber mutants in the sample only those auxotrophs with a reversion frequency approaching 10^{-6} were chosen for further study. Whitfield *et al.* (1966) also reported that almost all of the chain-terminator mutants (amber and ochre) were phenotypically suppressible by streptomycin. Only a minority of missense mutants and no frame-shift mutant shared this property. The *S. anatum* auxotrophs were, therefore, tested with streptomycin and only those mutants which proved suppressible were tested further.

Of twenty NG-induced auxotrophic mutants eight with appropriate reversion rates and suppressibility were investigated. Spontaneous revertants were tested for their ability to support growth of phage 01*am 1* by picking revertant colonies and streaking across a dried streak of phage suspension. Only revertant colonies that grew more or less as rapidly as wild type *S. anatum* on minimal medium were tested, because it was hoped to isolate strains carrying efficient suppressors.

Of the eight auxotrophs tested, at least five produced revertants capable of supporting growth of the mutant phage. From each of these five strains at least ten revertants sensitive to streaks of phage 01*am 1* were tested for their ability to form plaques from individual phage particles. Three of the strains produced no revertants capable of forming plaques with the mutant phage. Two of the auxotrophic strains produced significant numbers of revertants capable of forming plaques. One of these, an auxotrophic strain (designated Z) produced among its revertants a minority (4 of 58 tested) on which phage 01*am 1* formed small but distinct plaques.* On these strains, phage 01*am 4* formed plaques similar to those produced by the wild type phage. Both mutants had similar efficiencies of plating on these strains and upon the amber-suppressor strain of *S. typhimurium*.

These revertants, designated Zω (omega) strains, most probably contain amber-suppressor mutations. Since it is known that ochre suppressors will suppress amber

* Infection of the other revertants of strain Z did not consistently give rise to well defined plaques in the expected number. Some of these strains apparently carry suppressors not entirely suitable for suppression of the phage mutation but sufficient to allow significant leakage. These strains were not studied further.

mutants (though inefficiently) (Stretton *et al.* 1967) the possibility that they contain ochre suppressors has not been excluded. Probably, a definite conclusion will only be possible after the isolation of a large number of ochre and amber mutants of phage 01 and their testing against a range of suppressors in *S. typhimurium*.

Using one of the Zω strains it has proved possible to isolate a number of suppressor-sensitive (probably amber) mutants of bacteriophage ϵ^{15}.

The generality of this technique is of interest: the amber mutants of phage 01 could be used to identify amber suppressors in species of Salmonella and Arizona. Ochre and opel (UAA and UGA) mutants of phage 01 also could easily be produced. Other phages with a wider host range could be used for other genera. Some phages isolated from lysogenic *Proteus* species can grow on strains of *E. coli* (Coetzee, 1963), phages capable of growth on *E. coli* and *Serratia marcescens* are known (Wassermann & Seligmann, 1953; Bertani, Torheim & Laurent, 1967). These examples indicate that the method could be of general use in organisms with a very wide range of DNA compositions. Although the genera *Proteus*, *Escherichia* and *Serratia* are regarded as being related, their DNAs are very different. The GC contents are, respectively: 37–39·5 %, 50 % and 57·5 % (Marmur & Doty, 1962). Thus, phages are available to permit the identification of chain termination codons in organisms showing a wide range of DNA composition.

It may even be possible to use this technique to obtain nonsense suppressor mutations of higher organisms. It has recently been shown that the DNA of a phage of *Agrobacterium tumefaciens* can induce tumorous growth in angiosperms (Leff & Beardsley, 1970). The inducing DNA seems to be integrated into the DNA of the plant genome (Srivastava & Chadha, 1970). Nonsense mutation of the phage abolishing its ability to induce tumours may well prove suppressible by nonsense suppressor mutations of the plant.

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